

METHOD FOR REDUCING TOXICITY OF COMBINED CHEMOTHERAPIES**RELATED APPLICATION**

This application claims the benefit under Title 35 § 5 119(e) of U.S. Provisional Application No. 60/234,496, filed September 22, 2000.

FIELD OF THE INVENTION

This invention relates to the fields of oncology and 10 improved chemotherapy regimens.

BACKGROUND OF THE INVENTION

Taxol® (paclitaxel) has been shown to have excellent antitumor activity *in vivo*. Taxol® has been employed in 15 the treatment of a variety of cancers, including breast, ovarian and lung cancer.

The clinical use of doxorubicin (DOX), an anthracycline, in cancer therapy is limited by the possible development of chronic cardiomyopathy and 20 congestive heart failure, especially when the cumulative dose exceeds about 500 mg/m². Combining DOX with paclitaxel (PTX) has been shown to cause an unacceptable incidence of cardiac events at cumulative doses of the anthracycline below about 500 mg/m². Because of such 25 enhancement in cardiotoxicity, the cumulative dose of DOX must be reduced to about 360 mg/m² when the anthracycline is given in combination with PTX. There are reports indicating that combination therapy with docetaxel (DCT) would not increase the cardiotoxicity of DOX. It is 30 noteworthy, however, that very few patients in these studies were exposed to cumulative doses of DOX higher than about 360-400 mg/m².

Two-electron reduction of the carbonyl group in the side chain of DOX converts the anthracycline to a 35 dihydroxy (secondary alcohol) metabolite called doxorubicinol (DOXol). This reaction is catalyzed by NADPH oxidoreductases located in the cytosol. There is



growing evidence that DOXol may be an important mediator of cardiotoxicity. One-electron reduction of the quinone moiety in the tetracyclic ring of DOX generates a semiquinone that oxidizes to regenerate the parent
5 anthracycline by reducing oxygen to free radicals. This reaction is catalyzed by several reductases, including the microsomal NADPH cytochrome P-450 reductase and the mitochondrial NADH dehydrogenase. The role of free radicals in anthracycline-induced cardiotoxicity is
10 controversial, but these species may play a more definite role when synergized by the concomitant formation of DOXol.

As those experienced in the field of oncology appreciate, a need exists for combination chemotherapy
15 regimens having improved efficacy and safety.

SUMMARY OF THE INVENTION

In accordance with the present invention, a method for the treatment of cancer is provided comprising
20 administration of 4-desacetyl-4-methylcarbonate taxol and doxorubicin to a patient in need thereof. Surprisingly, it has been found that 4-desacetyl-4-methylcarbonate taxol does not stimulate formation of cardiotoxic metabolic doxorubicin by-products. In one embodiment of
25 the invention, the 4-desacetyl-4-methylcarbonate taxol and doxorubicin are administered to a patient sequentially in any order. In an alternative embodiment, the 4-desacetyl-4-methylcarbonate taxol and doxorubicin are administered concomitantly. Preferably, the agents
30 are administered sequentially with doxorubicin being administered first.

Also provided with the present invention is a chemotherapeutic composition comprising a chemotherapeutically effective amount of 4-desacetyl-4-
35 methylcarbonate taxol and doxorubicin. In a further embodiment of the invention, the chemotherapeutic composition is disposed within a pharmaceutically

acceptable carrier. Alternatively, each agent, 4-desacetyl-4-methylcarbonate taxol and doxorubicin may be formulated separately to facilitate sequential administration of the compositions.

5 The pharmaceutical compositions may be administered by any route and in any amount suitable to obtain the desired anti-cancer effect. In one embodiment, 4-desacetyl-4-methylcarbonate taxol and doxorubicin are separately infused into a patient with an interval
10 between infusions ranging from about 30 minutes to 24 hours with about a one hour interval being preferred. Intravenous administration of the agents is also preferred.

15

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram showing the metabolic conversion of doxorubicin to toxic by-products.

20 Figures 2A-2C depict the structures of paclitaxel (PTX) (Fig. 2A); docetaxel (DCT) (Fig. 2B) and 4-desacetyl-4-methylcarbonate taxol (Fig. 2C).

25 Figure 3 is a graph showing that PTX and DCT stimulate DOXol formation in cardiac cytosol. 4-desacetyl-4-methylcarbonate taxol did not stimulate DOXol production at the concentrations tested. * P= <0.05.

30 Figures 4A and 4B are graphs showing that PTX and DCT stimulate conversion of doxorubicinone (DOX aglycone hereafter) into doxorubicinolone (DOXol aglycone hereafter) (Fig. 4A). 4-desacetyl-4-methylcarbonate taxol failed to stimulate this conversion (Fig. 4B). * P= <0.025 vs. control.

35 Figures 5A and 5B are graphs showing that both PTX and DCT stimulate NADPH oxidation by deoxycholate-solubilized microsomes isolated from the human heart when

incubated with DOX (Fig. 5A). 4-desacetyl-4-methylcarbonate taxol stimulated this oxidation only at the highest levels tested (Fig. 5B). * P= <0.05 versus control.

5

Figure 6 is a graph showing that DOX enhances superoxide anion formation. Inclusion of 10 μ M PTX or DCT resulted in a significant increase in superoxide anion formation whereas the 4-desacetyl-4-methylcarbonate taxol did not. * P= <0.025 versus DOX; * * P= NS versus DOX.

10

DETAILED DESCRIPTION OF THE INVENTION

A systematic study was undertaken to characterize whether 4-desacetyl-4-methylcarbonate taxol increases DOXol and free radicals in the heart. Side-by-side comparisons of PTX, DCT and 4-desacetyl-4-methylcarbonate taxol were made. Studies have shown that a dose-related incidence of doxorubicin (DOX)-induced cardiotoxicity is increased by paclitaxel (PTX) but not docetaxel (DCT). It is possible however, that the reduced cardiotoxic effects of DOX-DCT combinations may be due to administration of lower cumulative doses of DOX. To characterize mechanisms and analogue-specificity of DOX-taxane interactions, an evaluation was undertaken to assess the effects of PTX, DCT and a new taxane 4-desacetyl-4-methylcarbonate taxol, on the metabolic conversion of DOX to toxic species in human myocardium. DOX metabolism was assessed in subcellular fractions of myocardial samples disposed of during aorto-coronary bypass grafting. The formation of DOX metabolites which mediate the acute and chronic phases of cardiotoxicity (oxygen free radicals, the aglyconic alcohol metabolite doxorubicinolone, and the glycosidic alcohol metabolite doxorubicinol, respectively) were measured. PTX and DCT (1 μ M) increase the cytoplasmic conversion of DOX and

doxorubicinone to doxorubicinol and doxorubicinolone, respectively [doxorubicinol: from 0.46 ± 0.04 to 1.1 ± 0.2 and 1.2 ± 0.2 nmol/mg prot./4h; doxorubicinolone: from 0.8 ± 0.1 to 1.4 ± 0.2 and 1.3 ± 0.2 nmol/mg

5 prot./4h; n=3, P <0.05). PTX and DCT (10 μ M) also increase the redox cycling of DOX with oxygen and the consequent generation of superoxide anion radicals in microsomal fractions (from 1.6 ± 0.1 to 2.2 ± 0.2 and 2.4 ± 0.2 nmol/mg prot./min, respectively; n=4-10, P< 10 0.05). Under comparable conditions 4-desacetyl-4-methylcarbonate taxol did not increase the formation of doxorubicinol, doxorubicinolone or superoxide anion radicals. As described herein, the commercially available taxanes PTX and DCT, but not 4-desacetyl-4-

15 methylcarbonate taxol, share the potential to increase levels of toxic DOX metabolites in human myocardium. While recommending cardiac surveillance in patients treated with DOX and PTX or DCT, these findings suggest less cardiotoxic potential when combining 4-desacetyl-4-methylcarbonate taxol with DOX. Advantageously, a higher cumulative dose of doxorubicin may be used in combination with 4-desacetyl-4-methylcarbonate taxol as compared to combinations of doxorubicin with PTX or DCT.

The enzymes of DOX metabolism are strongly species-related. To avoid the potential pitfalls associated with studies in animal models, an *in vitro* human heart assay was utilized. The assay involves isolation of both cytosolic fractions generating DOXol, and microsomal fractions converting DOX to free radicals.

30 Materials and methods for synthesizing 4-desacetyl-4-methylcarbonate taxol are provided in PCT/US93/12173 and EP 0604910B1, the entire disclosure of each of the above-identified patent applications being incorporated by reference herein. Pharmaceutically acceptable salts of 35 4-desacetyl-4-methylcarbonate taxol which also have reduced ability to stimulate formation of cardiotoxic by-products are within the scope of the present invention.

Such salts, including but not limited to, the hydrochloride or sodium salts, may be prepared following procedures which are familiar to those skilled in the art.

5 Methods for the administration of the chemotherapeutic agents of the invention are also disclosed. Doxorubicin and 4-desacetyl-4-methylcarbonate taxol may be administered to a patient simultaneously, or they may be administered sequentially in any order. In a
10 preferred embodiment, the anthracycline, doxorubicin, is administered initially. After approximately a period of about 30 minutes to 24 hours, 4-desacetyl-4-methylcarbonate taxol is administered. Dosages suitable for administration of the chemotherapeutic compositions
15 of the invention are set forth hereinbelow.

Whether administered sequentially or concomitantly, the compositions may be administered in any amount or by any route of administration effective for the treatment of cancer. Thus, the expression "chemotherapeutically effective amount", as used herein, refers to a sufficient amount of the compounds of the invention to provide the desired anti-cancer effect. The exact amount required will vary from subject to subject, the mode of administration of the chemotherapeutic compounds and the like.
25

The present invention further provides chemotherapeutic compositions comprising both 4-desacetyl-4-methylcarbonate taxol and doxorubicin. Alternatively, the chemotherapeutic pharmaceutical compositions of the present invention comprise one of the above-described compounds as the active ingredient, in combination with a pharmaceutically acceptable carrier medium or an auxiliary agent. Thus, in this embodiment, the 4-desacetyl-4-methylcarbonate taxol and the
30 doxorubicin are formulated separately.
35

The compositions of the pharmaceuticals of the invention may be prepared in various forms for

administration, including tablets, caplets, pills, or dragees, or can be filled in suitable containers, such as capsules, or, in the case of suspensions, filled into bottles. As used herein, "pharmaceutically acceptable carrier medium" includes any and all solvents, diluents, other liquid vehicle, dispersion or suspension aids, surface active ingredients, preservatives, solid binders, lubricants, and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Fifteenth Edition, E.W. Martin (Mack Publishing Co., Easton PA 1975) discloses various vehicles or carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of the invention.

In the pharmaceutical combination compositions of the invention, the active agents may be present in an amount of at least about 0.1% and not more than about 95% by weight, based on the total weight of the compositions, including carrier medium and auxiliary agent(s). Preferably, the proportion of active agent varies between about 1% and about 75% by weight of the composition. Pharmaceutical organic or inorganic solid or liquid carrier media suitable for enteral or parenteral administration can be used to make up the composition. Gelatine, lactose, starch, magnesium, stearate, talc, vegetable and animal fats and oils, gum, polyalkylene glycol, or other known excipients or diluents for medicaments may all be suitable as carrier media.

The chemotherapeutic compositions described herein are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit

form" as used herein refers to a physically discrete unit of chemotherapeutic composition for the patient to be treated. Each dosage should contain the quantity of active material calculated to produce the desired

- 5 therapeutic effect either as such, or in association with the selected pharmaceutical carrier medium. Typically, the anthracyclines, including doxorubicin, are administered in dosage units ranging between about 40 mg to about 200 mg of the agent, with a range of about 40-
10 150 mg being preferred. Typically, the taxanes, including 4-desacetyl-4-methylcarbonate taxol are administered in dosage units ranging between about 50-380 mg of the agent, with a range of about 50 mg to 300 mg being preferred. The chemotherapeutic combination
15 compositions of the invention may be administered orally, parenterally, by intramuscular injection, intraperitoneal injection, intravenous infusion, or the like. Intravenous administration is particularly preferred. The chemotherapeutic compositions of the invention are
20 typically administered by intravenous infusions of varying duration, with infusions of 1 hour to 24 hours being preferred.

The chemotherapeutic compositions may be administered one or more times every month to obtain the desired therapeutic effect. In a preferred embodiment of the present invention, the chemotherapeutic agents are sequentially administered by intravenous infusions, with doxorubicin being preferably administered first.

- Different intervals between administration of the
30 compounds may be selected. Such intervals may range between about 30 minutes and 24 hours, with 1 hour being preferred.

Biological studies of combination chemotherapeutic regimens have been performed in cardiac cellular fractions. Surprisingly, it has been discovered that 4-desacetyl-4-methylcarbonate taxol does not stimulate production of cardiotoxic metabolites and by-products of

doxorubicin when incubated with the anthracycline in human cardiac cytosol. The test procedures and results of these studies are set forth below.

5 **Reconstitution of DOX metabolism and DOXol formation, plus or minus taxanes, in human cardiac cytosol.**

Human myocardium is obtained from patients undergoing aorto-coronary bypass grafting. All samples
10 were routinely disposed of by the surgeons from the lateral aspect of excluded right atrium during cannulation procedures for cardio-pulmonary by-pass. After storage at -80 °C, pools of 15-20 samples are processed for cytosol preparation by sequential
15 homogenization, ultracentrifugation and overnight 65% ammonium sulfate precipitation of 105,000 g supernatants. Next, cytosol is dialyzed against 100 mM Tris HCl-40 mM KCl, pH 8.9, diluted to 3 mg protein/ml with the same buffer, and incubated for 15 minutes at room temperature
20 with 100 mM dithiothreitol to promote non-denaturing disassembly of the [4Fe-4S] cluster of cytoplasmic aconitase. This treatment is necessary as reactions of DOXol with [4Fe-4S] clusters would convert this metabolite back to DOX. Sizable amounts of DOXol would
25 therefore go undetected if they reacted with [4Fe-4S] clusters prior to their assay. Unreacted dithiothreitol was eventually removed by sequential gel filtration on (1.5 x 10 cm) Sepharose 6B columns, protein reprecipitation with 65% ammonium sulfate, and extensive
30 dialysis against 0.3 M NaCl, pH 7.0. Doxorubicin metabolism was reconstituted in 0.5 ml incubations containing cytosol (0.15 mg protein), NADPH (100 µM) and DOX (25 µM), in 0.3 M NaCl (pH 7.0) 37 °C. Where indicated ethanol-dissolved taxanes (1-50 µM) were included.
35 Aliquots of taxane-free ethanol (5 µl) were included as appropriate to adjust the final volume to 10µl in all incubations. After 4 h DOX and DOXol were extracted with

2 ml of CHCl₃/CH₃OH (1:1) and separated by previously validated two-dimensional TLC on (20x20 cm) 0.25 mM F524 Silica Gel Plates, using CHCl₃/CH₃OH/CH₃COOH/H₂O (80:20:14:6) as mobile phase in either dimension.

- 5 Doxorubicinol was identified by cochromatography with (S)-DOXol from Streptomyces peucetius or (S)(R)-DOXol purified after NaBH₄ reduction of DOX. Where indicated incubations were prepared by replacing DOX with DOX aglycone and by measuring formation of DOXol aglycone.
- 10 The latter was separated from DOX aglycone by using CHCl₃/CH₃OH/CH₃COOH (100:2:5) and CH₃COOC₂H₅-CH₃CH₂OH-CH₃COOH-H₂O (80:10:5:5) in the first and second dimension, respectively. DOX aglycone was purified after thermoacid hydrolysis of DOX.

15

Reconstitution of DOX metabolism and free radical formation, plus or minus taxanes, in human cardiac microsomes.

- 20 105,000 g pellets from human myocardium homogenates are solubilized with deoxycolate and reconstituted at the final concentration of 0.025 mg prot/ml with 10 µM DOX, 1-10 µM taxane, 0.1 mM NADPH, in 0.3 M NaCl, pH 7.0, 37°C.
- 25 Electron flow from NADPH cytochrome P-450 reductase to the quinone moiety of DOX is detected by monitoring NADPH oxidation. Redox coupling of the semiquinone with oxygen is detected by monitoring formation of the superoxide anion radical with the superoxide dismutase-inhibitable reduction of cytochrome c (25 µM). These assays are performed in a Hewlett Packard 8453A
- 30 UV/VIS/NIR spectrometer equipped with computer-assisted corrections for scatter and turbidity.

EXAMPLE 1**PTX AND DCT STIMULATE FORMATION OF TOXIC DOXORUBICIN
METABOLITES**

5 Both PTX and DCT stimulate DOXol formation in [4Fe-4S]-depleted human cardiac cytosol. As shown in Fig 3, the concentration-response curves were bell-shaped, suggesting that taxanes can act as allosteric modulators of anthracycline reductases. The range of concentrations 10 permissive to stimulation tends to be broader in the case of PTX; however, maximal stimulation by the two taxanes is identical in this set of experiments. 4-desacetyl-4-methylcarbonate taxol did not stimulate DOXol formation at any of the concentrations tested.

15 As shown in Fig. 4, PTX and DCT were also able to stimulate the conversion of DOX aglycone into DOXol aglycone. Again, the concentration-response curves were bell-shaped, the two taxanes being virtually identical with respect to both the net stimulation achievable and 20 the range of concentrations permissive to such stimulation (Panel A). 4-desacetyl-4-methylcarbonate taxol has not been found to stimulate the formation of DOXol aglycone from DOX aglycone (Panel B).

25 **EXAMPLE 2**

STIMULATION OF NADPH OXIDATION BY TAXANES

PTX and DCT were able to stimulate NADPH oxidation by deoxycolate-solubilized microsomes of human heart 30 incubated with DOX. In this system the concentration-response curves were not bell shaped, but the stimulation increased until it reached a plateau (Fig. 5A). 4-desacetyl-4-methylcarbonate taxol stimulated NADPH 35 oxidation only at the highest concentration tested, although this was not statistically significant. (Fig. 5B). None of the taxanes tested affected NADPH oxidation in the absence of DOX.

As shown in Fig. 6, DOX was able to enhance superoxide anion formation by deoxycolate-solubilized microsomes, consistent with its ability to shunt electrons from NADPH cytochrome P-450 reductase to molecular oxygen via reduction-oxidation of its quinone moiety. The inclusion of 10 µM PTX or DCT resulted in a significant increase in superoxide formation, that was consistent with the ability of these two taxanes to stimulate NADPH oxidation under comparable conditions.

Again, 4-desacetyl-4-methylcarbonate taxol did not afford any significant stimulation in these settings, which was consistent with the lack of significant effect on NADPH oxidation.

The results presented herein indicate that both PTX and DCT stimulate DOX metabolism in the human heart, resulting in increased formation of potentially cardiotoxic DOXol, DOXol aglycone, and oxygen free radicals like the superoxide anion. These findings provide molecular correlates to explain the increased cardiotoxicity of DOX-PTX combinations, and suggest that enhanced cardiotoxicity might occur also after DOX-DCT regimens if the cumulative dose of DOX were increased above about 360-400 mg/m². In contrast, 4-desacetyl-4-methylcarbonate taxol appears to lack this effect on DOX metabolism. Accordingly, 4-desacetyl-4-methylcarbonate taxol provides a superior taxane for use in combined chemotherapy regimens with DOX.

30

REFERENCES

Gehl J, Boesgaard M, Paaske T, Vittrup Jensen B, Dombernowsky P. *Combined doxorubicin and paclitaxel in advanced breast cancer: effective and cardiotoxic.* Ann Oncol 1996; 7: 687-93.

35

Gianni L, Munzone E, Capri G, Fulfarò F, Tarenzi E, Villani F et al. *Paclitaxel by 3-hour infusion in*

combination with bolus doxorubicin in women with untreated metastatic breast cancer: high antitumor efficacy and cardiac effects in a dose-finding and sequence-finding study J Clin Oncol 1995; 13:2688-99.

5

Gianni L, Dombernowsky P, Sledge G, Amadori G, Martin M, Baynes R et al. Cardiac function following combination therapy with Taxol¹⁷ and Doxorubicin for advanced breast cancer. Proc Annu Meet Am Soc Clin Oncol 1998; 17:115a
10 (abstract 444).

Nabholz JM, Smylie M, Mackey JR, Noel D, Paterson AH, al-Tweigeri T et al.

Docetaxel/doxorubicin/cyclophosphamide in the treatment of metastatic breast cancer. Oncology 1997; 11 (Suppl 8) : 37-41.

Olson RD, Mushlin PS. Doxorubicin cardiotoxicity : analysis of prevailing hypotheses. FASEB J 1990; 4: 20 3076-86.

Minotti G, Cairo G, Monti E. Role of iron in anthracycline cardiotoxicity : new tunes for an old song ? FASEB J 1999; 13: 199-212.

25

Boucek RJ Jr, Olson RD, Brenner DE, Ogumbumni ME, Inui M, Fleischer S. The major metabolite of doxorubicin is a potent inhibitor of membrane-associated ion pumps: a correlative study of cardiac muscle with isolated membrane fractions. J Biol Chem 1987; 262 : 15851-56.

Minotti G, Recalcati S, Liberi G, Calafiore AM, Mancuso C, Preziosi P et al. The secondary alcohol metabolite of doxorubicin irreversibly inactivates aconitase/iron regulatory protein-1 in cytosolic fractions from human myocardium. FASEB J 1998; 12 : 541-51.

- Minotti G, Cavaliere AF, Mordente A, Rossi M, Schiavello R, Zamparelli R. et al. *Secondary alcohol metabolites mediate iron delocalization in cytosolic fractions of myocardial biopsies exposed to anticancer anthracyclines.*
5 J Clin Invest 1995 ; 95 : 1595-1605.
Powis G. *Free radical formation by antitumor quinones.*
Free Radic Biol Med 1989 ; 6 : 63-101.

While certain preferred embodiments of the present
10 invention have been described and specifically
exemplified above, it is not intended that the invention
be limited to such embodiments. Various modifications
may be made to the invention without departing from the
scope and spirit thereof as set forth in the following
15 claims.